

#437

Apollo 11: Exposure of Lower Animals to Lunar Material

C. A. Benschoter, T. C. Allison, J. F. Boyd, M. A. Brooks, J. W. Campbell, R. O. Groves,
A. M. Heimpel, H. E. Mills, S. M. Ray, J. W. Warren, K. E. Wolf, E. M. Wood,
R. T. Wrenn and Z. Zein-Eldin

Apollo 11: Exposure of Lower Animals to Lunar Material

Abstract. Lunar material returned from the first manned landing on the moon was assayed for the presence of replicating agents possibly harmful to life on earth. Ten species of lower animals were exposed to lunar material for 28 days. No pathological effects attributable to contact with lunar material were detected.

Astronauts Armstrong, Aldrin, and Collins collected samples of lunar material on Apollo 11 mission and returned these samples to earth on 24 July 1969. Described here are the results of exposing selected species of fish and invertebrates to representative samples of lunar material for 28 days. The pooled sample used for the biological tests was composed of approximately 50 percent glass or glasslike material, contained from 0 to 10 parts per million (ppm) of indigenous organic material, and was free of water (1). Other tests revealed this sample to be only slightly soluble (about 2 ppm) in water.

Tests were designed to detect extra-terrestrial replicating agents possibly harmful to life on earth (2). No pathological effects or evidence of the presence of replicating organisms were detected in any of the exposed experimental animals. Neither daily observations of general health nor periodic histopathological examinations revealed deleterious effects attributable to contact with, or ingestion of, lunar material. Microscopic examination of the lunar sample utilized in these studies revealed that much of the lunar material was in the form of tiny beads. Ingestion of this material by the various species did not result in abrasion of the epithelial lining of the gastrointestinal tract.

The lunar material used in these studies was a portion of the pooled conventional sample (consisting of surface fines and rocks) that had been ground to a mean particle size of 2 μm . One-half of each sample was sterilized with dry heat at 160°C for 16 hours at ambient pressure before use.

Animals of ten species selected for

exposure to lunar material were maintained in class III glove cabinets inside the biological barrier system at the Lunar Receiving Laboratory. Optimum temperatures, photoperiods, feed, containers, and substrates were provided for each species to the extent possible.

Each species, with the exception of the protozoa and planaria, were divided into four test groups. At the time of inoculation, all test and control animals had been acclimated to the cabinet environment for as long as 2 weeks where appropriate. One group was inoculated with unsterilized material; a second group was inoculated with sterilized material; a third group was maintained within the cabinetry as an uninoculated control; and the fourth group was maintained in the normal animal colony as a cabinetry environment control group.

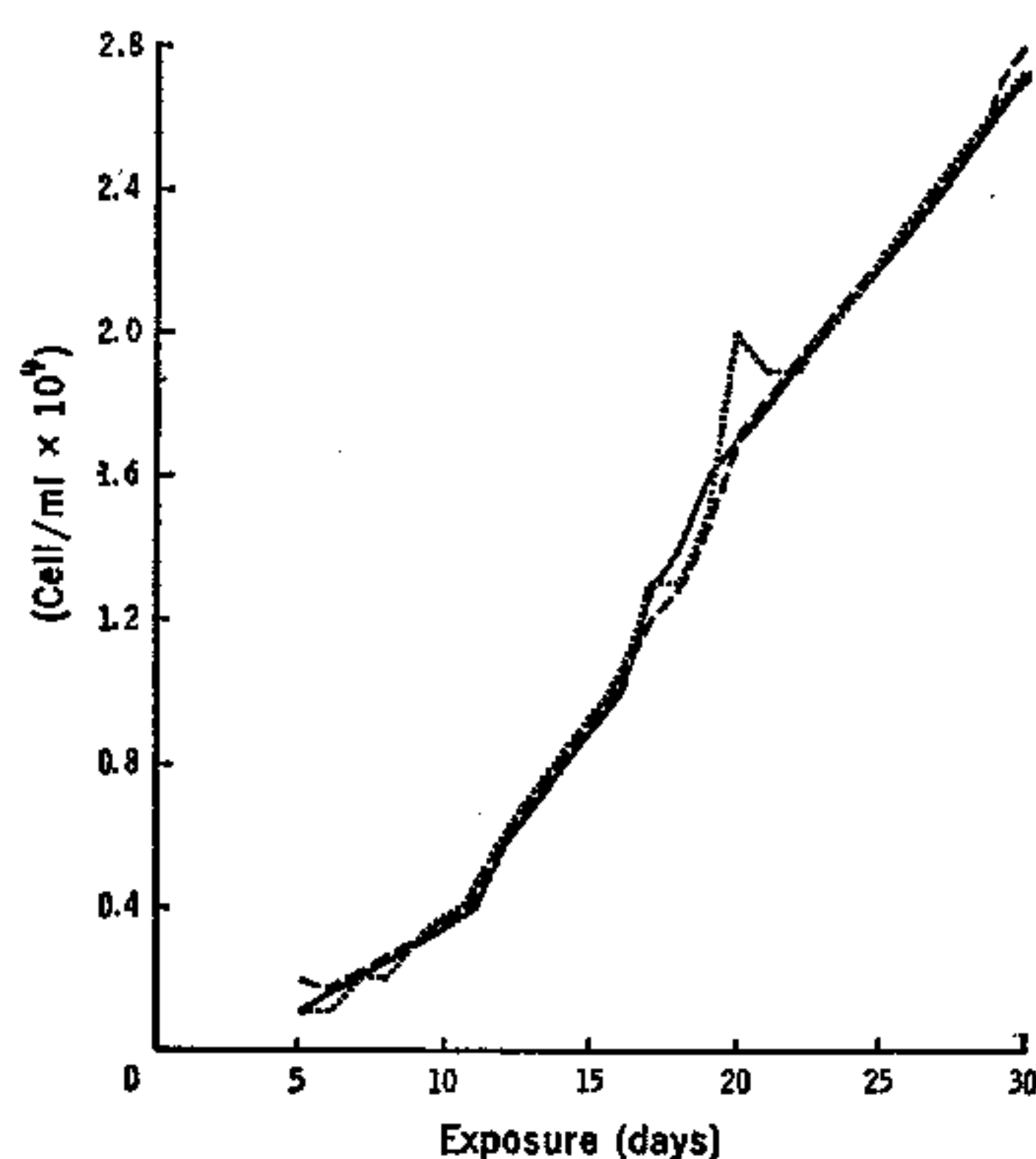


Fig. 1. Population growth in *Euglena gracilis*. Solid line, lunar sample; dashed line, sterilized lunar sample; dotted line, control.

Because of differences in cultural methods for aquatic and terrestrial species, the methods of providing exposure to the lunar samples differed (Table 1). The seven aquatic species were exposed by adding lunar material to the medium in which the animals were living. The three insect species were exposed by mixing the samples with their food.

Exposure of *Euglena gracilis* (Fig. 1) and *Paramecium aurelia* to lunar material did not affect their growth rates. During the first 3 days after exposure, the activity of exposed protozoans was subjectively judged to be less than that of the control cultures. Subsequently, locomotion by both species, the avoidance response in the *Paramecium*, and metabolism in the *Euglena* were judged to be normal. Permanent slides were prepared from samples collected on day 14 and day 28. Examination with the light microscope revealed no gross morphological changes.

No mortality or morphological changes occurred in any of the groups of planaria (*Dugesia dorotocephala*). For reasons that remain unknown, the animals in the fingerbowl inoculated with heat-sterilized lunar material traveled at the water surface more frequently than the animals in either of the other groups.

Daily observation and histopathological examination of German cockroaches (*Blattella germanica*) at 1 and 3 weeks showed these insects to be in excellent condition throughout the exposure period. The symbiotic bacteria in the fat bodies and ovaries were present in the normal distribution and abundance, and the gut lining (Fig. 2) was not abraded by the lunar material. There was also an apparent slight acceleration of the development of exposed cockroaches as compared with the control groups, which further indicated the lack of ill effects. However, these developmental differences were not statistically significant.

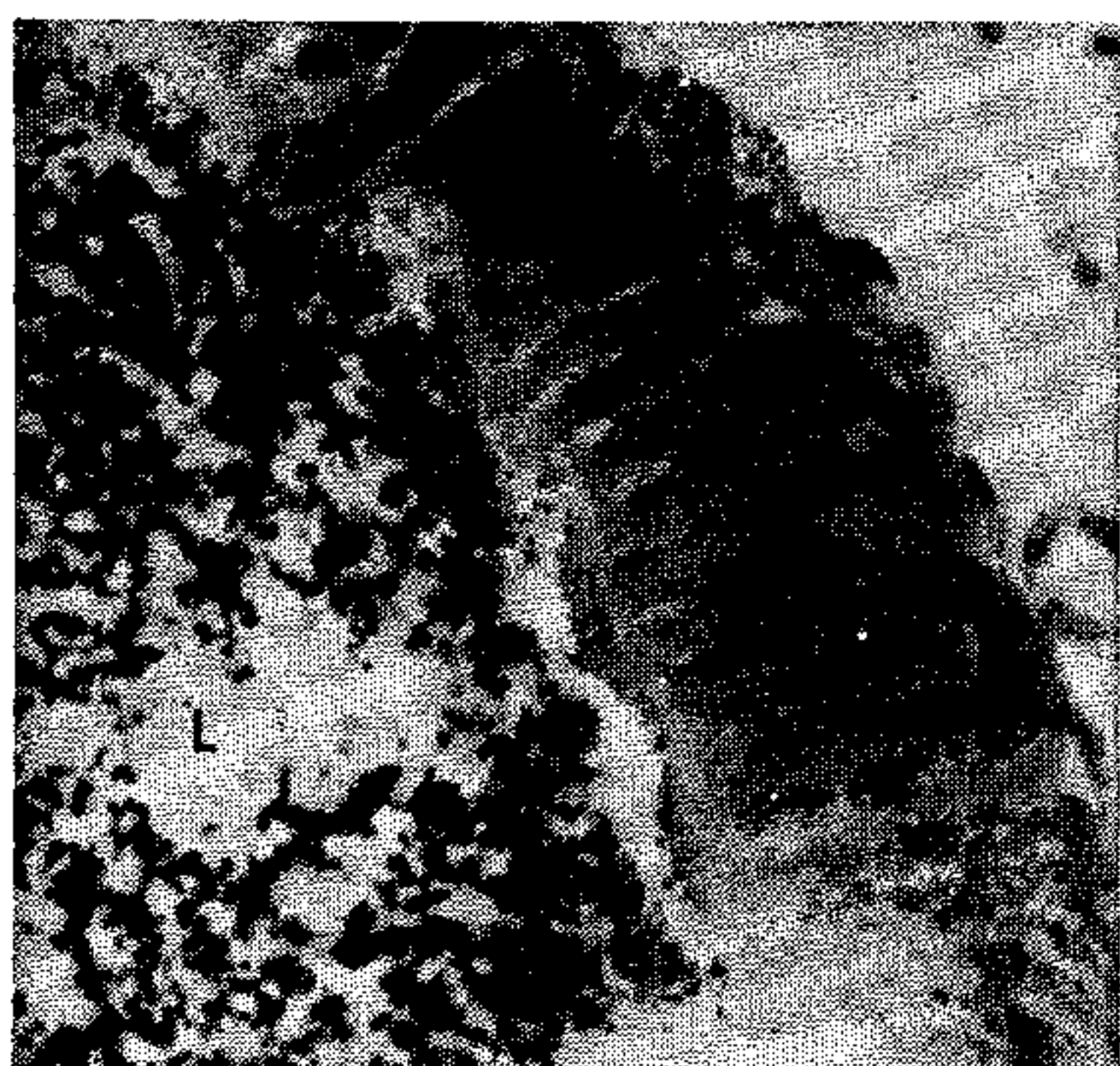


Fig. 2. Midgut epithelium (MG) of German cockroach with no evidence of abrasion by lunar material (L) in lumen.

Neither gross nor histopathological changes were detected in either the larval or the adult house flies (*Musca domestica*) exposed to lunar material. Several specimens from each group were killed after exposure for examination by both light and electron microscopy. No deleterious effects were found.

The response of greater wax moth (*Galleria mellonella*) larvae to lunar material in their diet was essentially the same as that of the other insects. Gross observations and histopathological findings showed the insects to be normal. Slightly higher survival ratios occurred in the lots exposed to lunar material, but these differences were not statistically significant.

Severe mortality occurred in all

groups of oysters (*Crassostrea virginica*). Deaths may have been due to the fact that the test was conducted during the spawning season and the animals' condition was poor. No unusual microorganisms or histopathological changes were detected in tissue sections prepared from control oysters or from those exposed to lunar samples. These histological examinations included oysters that became moribund or died during the 28-day exposure as well as the ten survivors killed and examined at the end of the test. Five of the survivors had been exposed to unsterile lunar material. Oysters from all groups were emaciated and completely "spawned out." There were no indications that exposure to lunar material was responsible for the high mortality.

Brown shrimp *Penaeus aztecus* exposed to the lunar samples showed no abnormal behavior or mortality. All body tissues were healthy, and the shrimp in all groups were in excellent physical condition throughout the test.

The fathead minnow *Pimephales promelas* is quite sensitive to toxic agents. Six days before exposure to lunar material, a spill of sodium hypochlorite in the laboratory caused the loss of approximately 50 percent of the fathead minnows being acclimated. Dead fish were replaced, but slight losses continued throughout the test. At day 14 and day 28 of the exposure period, histopathological examination of these specimens showed no indication of

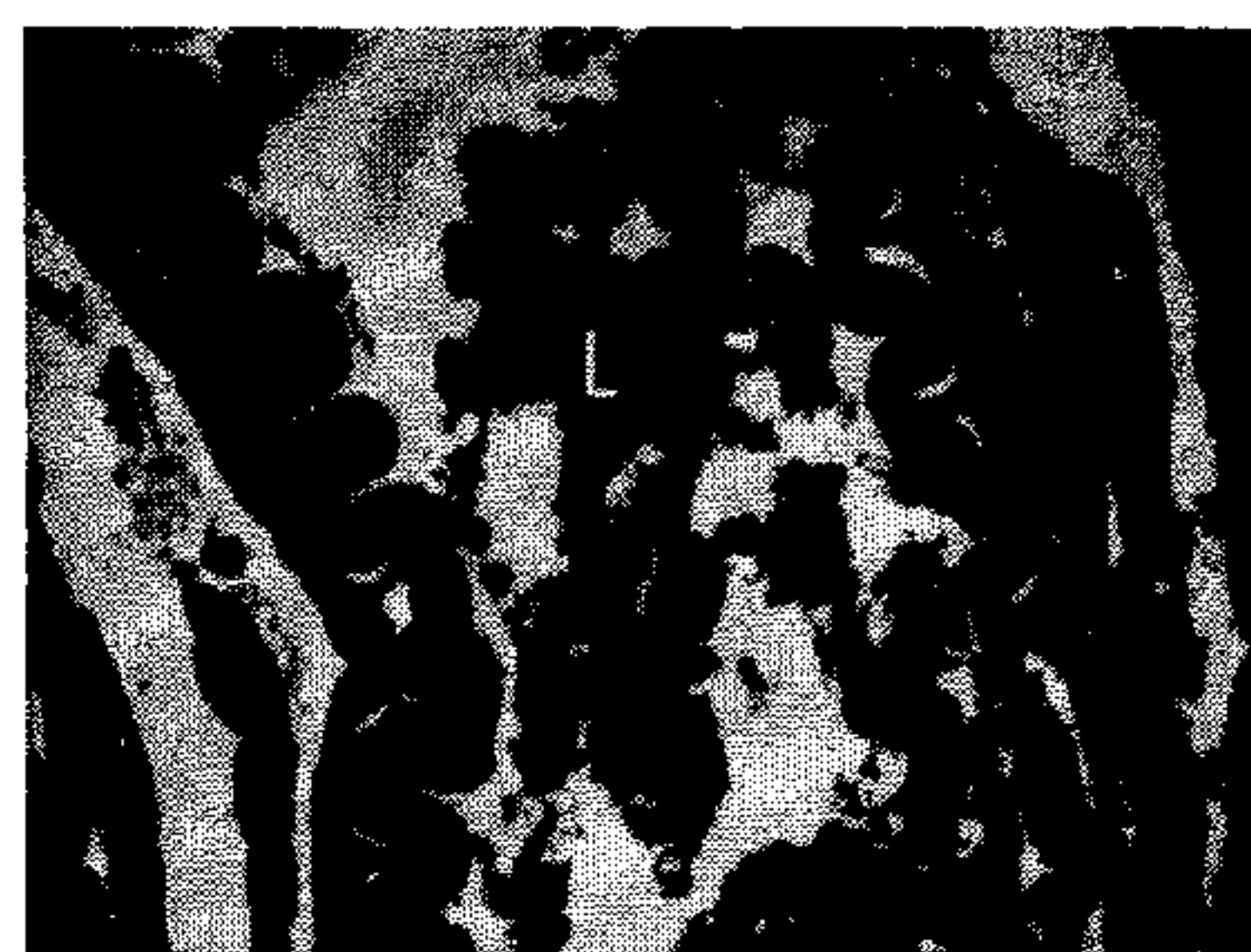


Fig. 3. Cross section of intestine of fathead minnow with no pathological effects caused by ingested lunar material (L) in lumen.

pathologic changes associated with their exposure to lunar material (Fig. 3). Residual pathology, which apparently represented long-term changes created by the hypochlorite spill, was noted in the gill tissues of some individual fish.

No pathologic changes of an unknown or unidentifiable character were noted during daily observation of behavioral and morphological features in the mummichog *Fundulus heteroclitus*. This marine minnow appeared to be especially hardy, and exposed groups appeared to be unaffected by the presence of the lunar material or by the spill of sodium hypochlorite in the laboratory. Histopathological examinations of sampled mummichogs at 14 and 28 days indicated that these fish were in excellent condition.

The nature of the lunar samples to

Table 1. Test facilities, environmental conditions, and lunar sample inoculation data.

Organism	Type of container	Number of containers per test group	Number of animals per container	Total containers	Diet	Medium	Photoperiod (hours light/hours dark)	Temperature (°C)	0.220 g lunar material per:
<i>Euglena</i>	250-ml flask	16	1.0×10^3 to 2.8×10^4	48	Provided by medium	Inorganic medium	16/8	22	Flask
<i>Paramecium</i>	125-ml flask	25	1.0×10^3 to 1.6×10^4	75	<i>Aerobacter aerogenes</i>	Lettuce leaf infusion	16/8	22	Flask
Planaria	300-ml finger-bowl	1	50	3	Calf liver	Aged tap water	16/8	22	Bowl*
Brown shrimp	20-liter aquarium	2	20	8	Live brine shrimp	Seawater 25 ppt	16/8	27	Shrimp
Oyster	1-liter jar	10	1	40	None	Instant ocean 21 ppt	16/8	22	Oyster
German cockroach	500-ml jar	3/3†	20/15	9/9	Mouse diet‡		16/8	25	Jar
House fly	500-ml jar	3/2§	25/25	9/6	Compounded diet		16/8	25	Jar
Greater wax moth	500-ml jar	3	25	9	Compounded diet ¶		Total darkness	25	Jar
Fathead minnow	20-liter aquarium	2	20	8	Live brine shrimp	Aged tap water	16/8	22	Fish
Mummichog	20-liter aquarium	2	20	8	Live brine shrimp	Instant ocean 21 ppt	16/8	22	Fish

* 3.30 g per bowl. † Nymphs/adults. ‡ Charles River prefortified rat-mouse diet. § Larvae/adults. || Sugar, albumen, sodium oleate, Wesson salts, B vitamins. ¶ Pabulum, sugar, glycerol, water, B vitamins.